

PB-237 889-T

INDUCTION OF HEPATIC TUMORS IN MICE
BY BENZIDINE

O. G. Prokofeva

Environmental Protection Agency
Research Triangle Park, North Carolina

1974

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE

308071

FROM: SCITRAN, SANTA BARBARA, CALIF. Phone: (805) 969-2413

TO: ENVIRONMENTAL PROTECTION AGENCY

ATT'N: CHANDLEE MURPHY

Phone: (919) 688-8146, Ext. ~~591~~

EPA-TR. 111-74

591

PB 237 889 -T

INDUCTION OF HEPATIC TUMORS IN MICE BY BENZIDINE

O.G. Prokof'yeva

It is well known from the literature that the blastomogenic action of benzidine, which is of great cancerogenic danger to man and has caused in him bladder tumors, has been experimentally verified with animals [4]. In biological experiments, it has been found that rats are most sensitive to the cancerogenic action of benzidine. Malignant neoplasms have been discovered in rats, localized in the liver, intestines, and hypodermic tissue at the location where the substance was directly applied. However, not in one case was the development of tumors in the bladder observed [3, 6 - 8].

Tumors of the bladder developed in dogs under the influence of benzidine, but in a small percent of the cases and many years after the beginning of the experiment [7, 8].

There are very brief reports on the blastomogenic action of benzidine upon mice in the literature. Only one study by Bonser, et al. [5] cites individual cases of the development of hepatic tumors in mice after the subcutaneous injection of benzidine. We performed the present experiments to obtain more detailed information on the action of benzidine upon mice.

In the experiment, we used 181 mice of line C3HA (168 males and 13 females), weighing 18-20 grams, obtained from the Rappolovo nursery. Once every 7 days benzidine was introduced under the skin of the mice in an amount of 6 mg (per 0.2 ml 3% butyric solution of the substance). In the first series of mice (54 males and 13 females), the substance was introduced for 8 months (total dose of 210 mg), and in the second series (114 males) - for 13 months (total dose of 336 mg). The mice who died or were sacrificed during the experiments were dissected, the organs and tissues with pathological changes were fixed in 10% Formalin, the sections were colored with hematoxylin-eosin, and sudan III was used according to the Van Gizon method (with saturn red) in individual cases to reveal any adipose inclusions.

As may be seen from the table on the next page, by the 16th month from the beginning of the experiment, the first tumor was discovered in both series in 46 mice (42 males and 4 females). By the 28th month, tumors were discovered in 32 mice (29 males and 3 females); they had developed in the lungs in 2 and in the liver in 31 mice (28 males and 3 females). An increase in the dose of benzidine (Series II) somewhat increased the frequency and greatly reduced the latent period for the development of neoplasms.

The clearly expressed toxicity of benzidine, which was very apparent in the first months of the experiment, must be noted. When the sacrificed animals were dissected, degenerate changes were found in the parenchymatous organs, and in several cases edema and hyperemia of the hypodermic tissue

were observed at the place where benzidine was introduced.

In the later parts of the experiment (from 15 to 28 months), the liver of several mice displayed large single, or small multiple, nodules, which as a rule developed on the lower surface of the organ. In two cases, small * neoplasms were found in the lungs of the mice.

A microscopic study of the experimental material revealed certain non-specific changes in the liver of mice who died in the early periods of observation. These changes consisted primarily of extensive infiltration of lymphoid cells, considerable expansion of the lumen of the vasa, and an increase in the number of nucleated cells of the parenchyma. Frequently changes were observed which are characteristic of adipose distrophy of the liver. In the mice who died in the later portions of the experiment, moderate proliferation of the mesenchymous elements was observed. In the liver of some mice, segments of the tissue were found which consisted of small, uniform parenchymatous cells separated from the liver tissue by a thin connective tissue layer or liver cells of elongated form. Such neoplasms may be assumed to be malignant adenomas of the liver.

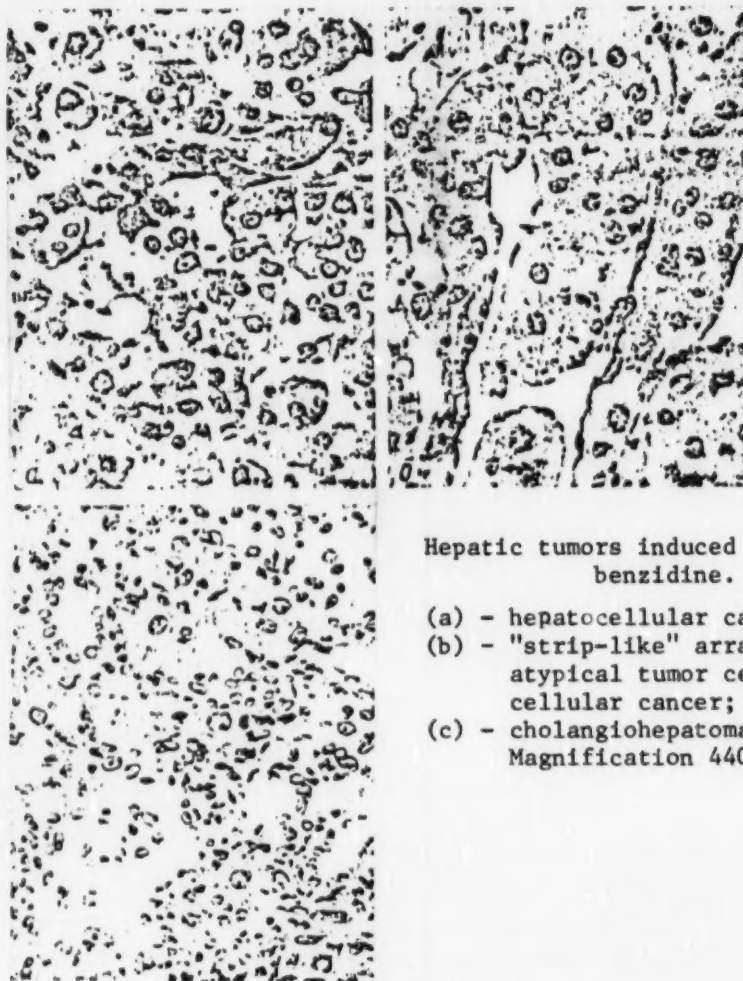
RESULTS OF EXPERIMENTS ON INTRODUCTION OF BENZIDINE

Series of Experiments	No. of injections	Total dose, mg	Amount		
			In exp't	up to appearance of 1st tumor	with tumors
I	35	210	67	22	14
II	56	336	114	24	18
Total		-	181	46	32

Series of Experiments	Detection of tumors by months										Localization of tumors	
	17	18	19	20	21	22	23	24	25	26	Liver	Lungs
I	-	1	1	1	5*	-	2	1	1	1	13*	2
II	4	2	5	1	1	3	-	1	1	1	18	-
Total	4	3	6	2	6*	3	2	1	1	1	31*	2

*In one mouse combined with a tumor of the lungs

*Translator's note: Illegible in foreign text.



Hepatic tumors induced in mice by benzidine.

- (a) - hepatocellular cancer;
 - (b) - "strip-like" arrangement of atypical tumor cells of hepatocellular cancer;
 - (c) - cholangiohepatoma.
- Magnification 440.

REFERENCES

1. Malyugina, L.L., O.G. Prokof'yeva. Voprosy onkol., 1957, 3, 2, 197.
2. Medvedev, N.N. Prakticheskaya genetika (Practical Genetics), Moscow, 1966, 221, 227.
3. Pliss, G.B. Voprosy onkol., 1964, 10, 5, 50.
4. Temkin, I.S. Opukholi mochevogo puzrya, vyzvannye kantserogennymi aminocoyedineniyami (Bladder tumors caused by cancerogenic amino compounds), Moscow, 1962.
5. Bonser, G.M. Brit. J. Cancer, 1936, 10, 3, 533.
6. Rhoads. In: Hartwell, Survey of Compounds which have been Tested for Carcinogenic Activity. Washington, 1951.
7. Spitz, S., Maguigan, W., Dobriner, K. Cancer, 1950, 3, 789.
8. Spitz, S. (cited by Walpole, A.L., et al.) Brit. J. Industr. Med., 1954, 11, 105.

Almost all of the hepatic tumors were hepatocellular cancer of differing morphological structure. Some of them consisted of extensive sections of parenchymatous cells with adipose degeneration, and a group of islands of extremely atypical cells, which were remotely similar to liver cells. Cystic formations of differing dimensions (pseudocysts) were frequently observed in the tumor tissue, which were filled with a serous liquid. Many of the tumors were in the form of individual islands of polymorphic cells (Figure, a) or in the form of strip-like complexes of atypical hepatic cells with clearly expressed nuclear polymorphism (Figure, b). In individual cases, there were tumors of the cholangioma type (Figure, c) consisting of small cysts lined with cells similar to the epithelium of bile ducts and small segments of atypical parenchymatous cells.

All of the neoplasms studied displayed infiltrating and destructive growth. Attempts were made twice to transplant the tumor tissue to mice-recipients. The attempts were successful, and one of the strains has continued until recently (18th generation).

Tumors of the lungs, which were discovered in 2 mice, displayed malignant adenocarcinomas. Thus, after subcutaneous introduction of benzidine in mice of the C3HA line, tumors developed in 69.5% of the cases. In 67.4% of the mice there were hepatomas of differing morphological structure.

These studies were performed on mice of the C3HA line which were sensitive to hepatotropic cancerogens. According to reports of several authors, the spontaneous development of hepatomas was observed in 1% at the most of C3HA mice [1]. N.N. Medvedev [2] published data indicating only a certain predisposition toward spontaneous development of hepatomas. A comparison of the results of our studies with the indices of tumors developing spontaneously in mice of the C3HA line reveals the clearly expressed hepatotropic action of benzidine. Tumors of the lungs, which were only detected in individual mice in the experiment, did not exceed the number of spontaneously developing neoplasms at this location. Important biological differences are revealed when comparing these results with data obtained by researchers on other types of animals. Thus, introducing benzidine to dogs causes the development of tumors in the bladder, whereas in rats neoplasms arise at different locations, including the place where the benzidine was injected. Subcutaneous injection of benzidine in mice of the C3HA line led to the formation of hepatic tumors, whereas the introduction of the preparation in the hypodermic tissue did not in one case lead to the development of any neoplasms at the location where it was introduced. The differing blastomogenous action of the same cancerogen in different kinds of animals is observed in experiments comparatively frequently. The assumption formulated by many researchers stipulating that there is a difference in cancerogen metabolism must be used to explain this.